Inter-Regional Differences in Baseline Toxicity of *Bemisia argentifolii* (Homoptera: Aleyrodidae) to the Two Insect Growth Regulators, Buprofezin and Pyriproxyfen

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ABSTRACT A survey of 53 *Bemisia argentifolii* Bellows & Perring populations from different agricultural regions in California and Arizona was conducted from 1997 to 1999 to establish baseline toxicological responses to buprofezin and pyriproxyfen. Although both compounds proved to be highly toxic even in minute quantities to specific stages, geographical and temporal differences in responses were detected using a leaf spray bioassay technique. Monitoring for three years revealed that six to seven populations had higher LC_{50} values but not greater survival when exposed to these two insecticides. A significant difference in relative susceptibility to buprofezin was first observed in late season 1997 in San Joaquin Valley populations with LC_{50}s ranging from 16 to 22 mg (AI) / liter^{-1} compared with LC_{50}s of 1 to 3 mg (AI) / liter^{-1} in Imperial, Palo Verde Valley and Yuma populations. Whiteflies collected in subsequent years from these and other locations showed an increase in susceptibility to buprofezin. Regional differences in susceptibilities to pyriproxyfen were minimal within the same years. Three years of sampling revealed consistently higher LC_{50}s to pyriproxyfen in populations from Palo Verde Valley, CA, compared with whiteflies from Imperial, San Joaquin Valley or Yuma. As was the case with buprofezin, a decline in LC_{50}s to pyriproxyfen was observed in whiteflies from all locations sampled in 1999. However, no correlation was observed between buprofezin and pyriproxyfen toxicity in any of the strains. The variable toxicities observed to both compounds over a period of 3 yr may be due principally to inherent differences among geographical populations or due to past chemical use which may confer positive or negative cross-resistance to buprofezin or pyriproxyfen.

KEY WORDS *Bemisia argentifolii*, whiteflies, resistance monitoring, baseline susceptibility

UNDER THE CURRENT control programs for the whitefly, *Bemisia argentifolii* Bellows & Perring, the combined cost of control on agricultural and floricultural crops is estimated to be approximately $1 billion in the United States. Suppression of the high numbers of *B. argentifolii* populations on various crops is still accomplished with insecticides. Concerns about insecticide resistance development to conventional insecticides has generated considerable interest in the use of chemicals with novel modes of action. Novel chemistries can be integrated into control programs to provide efficacious pest management and reduce resistance risk. The use of insecticide rotations is an effective strategy for delaying resistance development (Georghiou 1983, 1994) and it is therefore important to integrate these new chemicals into resistance management programs to delay the evolution of resistance.

One group of chemistries that has great potential for reducing broad scale insecticide use is comprised of the insect growth regulators (IGRs). Their unique mode of action is very selective and insect-specific (Miyamoto et al. 1993). These compounds are slow acting against a narrow range of sensitive stages of the insect's life cycle with high potency against target pests (Casida and Quistad 1999). Two new IGR insecticides, buprofezin (Applaud 70 WP) and pyriproxyfen (Knack 0.86 EC), have been introduced into California and Arizona agriculture under section 18 legislation. Buprofezin, an inhibitor of chitin synthesis is active against the immature stages of whiteflies. It acts by disrupting molting of larval stages to the adult stage (Ishaaya et al. 1988). Pyriproxyfen is a juvenile hormone analog that inhibits juvenile hormone (JH) synthesis in the corpora allata thus simulating the activity of endogenous JH (Baker et al. 1986). Pyriproxyfen is known to be active against many species of insects including mosquitoes, houseflies, aphids, greenhouse whitefly and pear psylla (Mulla et al. 1986, Iwanaga and Kanda 1988, McMullen 1990, Hatakoshi 1992). It has ovicidal and sterilant activities and is toxic to sweetpotato whitefly (*Bemisia tabaci* Gennadius) eggs by suppressing embryogenesis, but is not known to be acutely toxic to adults (Ishaaya and Horowitz 1992, Horowitz et al. 1994, Ishaaya et al. 1994). Both buprofezin and pyriproxyfen are extremely effective in suppression of whitefly numbers when applied at an appropriate stage. These two IGRs may be useful in

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maintaining whitefly infestation rates below economic injury levels. Reduction of field infestations early in the crop season by these would enhance the efficacy of other products later in the season due to the presence of parasitoids and other natural enemies.

An inevitable limitation in the use of any new class of insecticide is the development of resistance over time. Resistance to newly registered products is of particular concern because they are often more expensive than older insecticides due to their higher cost of development in the modern and more demanding regulatory environment. Moreover, new product development has slowed considerably relative to decades past, therefore requiring greater stewardship to conserve their activities. The susceptibility of an insect population to newer insecticides can be influenced by the past history of exposure to insecticides (Georghiou and Taylor 1986). The risk of resistance to new products can also be expected to increase where continuous long-term use of a product is practiced. Resistance monitoring surveys that are capable of detecting shifts in insect responses can play an important role in the long-term management of insecticide resistance. The early detection of resistance potentially enables counter-resistance measures to be taken in conjunction with any strategy that may already be in practice as part of an insecticide resistance management (IRM) program.

The novel chemistries represented by buprofezin and pyriproxyfen mandated that wide-ranging surveys of *B. argentifolii* populations be conducted to establish a response range to each product before their widespread use. Quantitative information on differential toxicity obtained in a monitoring program is crucial for validating and fine-tuning resistance management strategies for this pest.

In this study, the baseline susceptibilies of the immature and egg stages of several natural populations of whiteflies from California and Arizona to buprofezin and pyriproxyfen, respectively were measured. The initial and subsequent determination of dose-mortality relationships of the two IGRs against whiteflies will be a measure of changes in efficacy of the two products as they are being used with increasing frequency over time. The time frame of this study was three years.

Materials and Methods

Insects. Populations of adult whiteflies collected from cotton, melon and broccoli fields in Imperial, Palo Verde, San Joaquin Valleys in California and Yuma, AZ, were tested for their susceptibility to buprofezin and pyriproxyfen from 1997 through 1999. Collections were made at various times from July through December of each year to include mid- and late season cotton and early season melons and broccoli. The total number of sites that were sampled in the four agricultural regions varied from year to year. In Imperial Valley, 4, 6, and 12 sites were selected for sampling in 1997, 1998, and 1999, respectively. Eleven fields were sampled in Palo Verde Valley from 1997 to 1999. An average of three field sites per year were selected in San Joaquin Valley for this study. Additionally, whiteflies from four and three sites in Yuma, AZ, were included for comparison of susceptibility to the two IGRs during 1998 and 1999, respectively. Adults were collected by vacuuming on the foliage of the various crops. The adults were transported to the laboratory in wooden transfer cages on cotton plants for testing. Susceptibility of these whiteflies to buprofezin and pyriproxyfen was determined using the leaf spray bioassay described below.

Adult whiteflies collected from both states have been exposed to a number of insecticides including pyrethroids, organophosphates, and imidacloprid for a number of years. However, the comparison of cross-resistance patterns between the two IGRs and conventional insecticides in the wild populations of whiteflies was not the goal of this study, so the history of past insecticide use against these populations was not considered here.

Insecticides. Formulated grade samples of buprofezin (Applaud) and pyriproxyfen (Knack) were obtained from AgrEvo USA Company (Wilmington, DE) and from Valent (Walnut Creek, CA), respectively. Preliminary studies were performed with each compound to determine the appropriate concentration ranges for testing. Serial dilutions of these two materials to the desired five to six concentrations were made in water on the same day of application. Units for these two compounds are presented in mg (AI)/liter^{-1}.

Bioassay Techniques. Buprofezin bioassay. Immature susceptibility to buprofezin was determined in bioassays on cotton, *Gossypium hirsutum* 'Deltapine '5415', plants in the two true-leaf stage as described in Toscano et al. (1998). Forty unsexed adult whiteflies were confined on individual attached cotton leaves in clip cages of 8 cm^2 for 24 h to allow oviposition to obtain atleast 30 eggs but usually obtained more. Adults were removed from the infested leaves after 24 h. Egg-infested plants were maintained in whitefly-free cages within a growth chamber at 27°C, 30–40% RH, and a photoperiod of 12:12 (L:D) h. Seven days after egg deposition, sessile first instar nymphs were counted before treatment. Not all the eggs laid hatched so the groups of first instars that were treated by various concentrations varied from leaf to leaf with a minimum of 25 but usually around 40–50. Leaves infested with first immature stage were then sprayed to run-off with various concentrations of buprofezin. Five to six concentrations resulting in 10–95% mortality were selected and sprayed for each test. Each treatment was replicated three to four times. Control plants were sprayed with water alone. Treated plants were maintained in whitefly-free cages in a greenhouse (28 ± 3°C) to allow time for development. Immature mortality was assessed by counting the third and fourth stage nymphs that were alive on day 16 after oviposition. Insects were considered dead if they were dry and could be separated easily from the leaf surface. The number alive was deducted from the total

Table 1. Baseline monitoring of field populations of whiteflies from California to buprofezin in 1997

Location	Field#	Date/Year	*n*	Slope ± SE	LC_{50} (mg [AI]/l^{-1}) (95% CI)	χ^2 (df)
Imperial	1	July '97	1,148	1.5 ± 0.11	4.57 (1.92–11.76)	6.64 (5)
Imperial	2	July '97	792	1.1 ± 0.44	1.31 (0.09–10.54)	8.91 (4)
Imperial	3	Sept '97	574	2.2 ± 0.58	4.34 (1.23–9.12)	10.79 (5)
Imperial	4	Sept '97	624	1.4 ± 0.25	2.12 (0.53–12.47)	8.26 (5)
Imperial	5	Oct '97	946	1.1 ± 0.40	2.74 (0.06–16.38)	7.74 (4)
Palo Verde	1	Aug '97	670	1.1 ± 0.20	2.68 (0.21–17.85)	10.82 (5)
Palo Verde	2	Aug '97	698	1.2 ± 0.17	1.88 (0.11–11.85)	6.13 (4)
Palo Verde	3	Sept '97	583	1.4 ± 0.18	2.01 (0.84–15.31)	5.66 (5)
San Joaquin	1	Aug '97	378	2.3 ± 0.13	22.28 (14.21–29.2)	9.25 (5)
San Joaquin	2	Aug '97	380	2.8 ± 0.23	37.14 (28.33–41.0)	8.43 (5)
San Joaquin	3	Sept '97	360	2.4 ± 0.27	14.85 (10.28–24.3)	10.18 (5)
San Joaquin	4	Sept '97	340	1.2 ± 0.34	9.55 (1.89–19.7)	6.96 (4)
San Joaquin	5	Oct '97	458	1.5 ± 0.31	3.88 (0.93–14.22)	9.39 (5)
San Joaquin	6	Oct '97	536	1.4 ± 0.30	1.19 (0.09–10.57)	5.12 (4)

number of first stage nymphs that were recorded on day 7 before treatment to assess mortality rates.

Pyriproxyfen Bioassay. Cotton leaves were infested with eggs as described with buprofezin by confining forty adults per leaf in the clip cages for 24 h. The total number of eggs on each leaf was counted before the application of pyriproxyfen. Leaves that were infested with a minimum of 30 eggs were used for the tests. Leaves with fewer eggs than 30 were discarded. Leaves were sprayed to run-off on the same day with five to six concentrations of pyriproxyfen ranging from 0.5 to 323 mg (AI)/$liter^{-1}$. In most cases, six concentrations plus a water control were replicated three times against each population.

Treated plants were maintained in whitefly-free cages in a growth chamber at 27°C, 30–40% RH, and a photoperiod of 12:12 (L:D) h to allow development of the immatures. Egg mortality was assessed 7–8 d after treatment by subtracting the number of live first stage nymphs from the total number of eggs laid. Total mortality was calculated by subtracting the number of first stage nymphs alive from the total number of eggs laid.

Statistical Analysis. Bioassay data on the mortality of the immatures exposed to the two IGRs were pooled and analyzed based on standard probit analysis as adapted to personal computer use by the POLO program (LeOra Software 1987). Differences in LC_{50}s between populations were considered significant (<0.05) when 95% confidence intervals did not overlap. Data for 3-yr comparison for each compound was analyzed deriving treatment means for each of the three trials and by using the three means in one-way analysis of variance (ANOVA) using the statistical package for the Macintosh (SAS Institute 1994) with means separated by the Tukey-Kramer honestly significant difference (HSD) test ($0 < .05$). Data for this part is presented as means ± SEM graphically.

Results

Baseline Susceptibility to Buprofezin. Buprofezin susceptibility results for all whitefly populations sampled between 1997, 1998 and 1999 are presented in Tables 1–3. Although all the populations tested appear to be highly susceptible (based on long-term survival) to buprofezin, there were some significant regional differences detected. The range of sensitivity varied by as much as 1,238-fold with some significant probit regression estimates during the 3 yr of sampling (Table 1–3). The LC_{50} values ranged from 0.03 to 37.14 mg (AI)/$liter^{-1}$ for the whiteflies from the three valleys

Table 2. Baseline monitoring of field populations of whiteflies from California and Arizona to buprofezin in 1998

Location	Field #	Date/Year	*n*	Slope ± SE	LC_{50} (mg [AI]/l^{-1}) (95% CI)	χ^2 (df)
Imperial	1	Aug '98	1,157	3.5 ± 0.09	0.24 (0.10–1.85)	10.19 (5)
Imperial	2	Aug '98	569	2.1 ± 0.25	3.11 (0.86–10.24)	6.43 (5)
Imperial	3	Sept '98	453	2.0 ± 0.19	2.97 (1.01–12.45)	8.21 (5)
Imperial	4	Sept '98	621	1.1 ± 0.17	1.56 (0.35–13.92)	7.13 (4)
Imperial	5	Oct '98	636	1.3 ± 0.22	1.15 (0.36–11.29)	8.82 (5)
Imperial	6	Nov '98	733	1.9 ± 0.13	1.66 (0.67–10.89)	7.56 (5)
Palo Verde	1	Aug '98	384	1.0 ± 0.45	2.75 (0.83–18.78)	10.81 (5)
Palo Verde	2	Aug '98	671	1.5 ± 0.25	0.32 (0.07–4.21)	7.73 (5)
Palo Verde	3	Oct '98	456	1.0 ± 0.52	0.86 (0.04–6.36)	6.41 (4)
San Joaquin	1	Aug '98	363	1.1 ± 0.41	1.61 (0.08–11.72)	9.32 (5)
San Joaquin	2	Aug '98	476	1.6 ± 0.27	3.21 (0.91–8.34)	6.14 (4)
San Joaquin	3	Sept '98	1348	1.3 ± 0.38	0.43 (0.05–5.78)	5.56 (5)
Yuma	1	Aug '98	751	1.2 ± 0.24	3.25 (0.89–10.45)	4.48 (4)
Yuma	2	Aug '98	533	1.1 ± 0.31	1.87 (0.07–8.73)	10.75 (5)
Yuma	3	Sept '98	1,122	2.5 ± 0.22	1.25 (0.08–5.83)	9.87 (5)
Yuma	4	Sept '98	893	2.1 ± 0.29	2.76 (0.79–6.33)	7.61 (5)

Table 3. Baseline monitoring of field populations of whiteflies from California and Arizona to buprofezin in 1999

Location	Field #	Date/Year	*n*	Slope ± SE	LC_{50} (mg [AI]/l^{-1}) (95% CI)	χ^2 (df)
Imperial	1	June '99	429	1.2 ± 0.21	0.17 (0.02–4.61)	7.60 (5)
Imperial	2	June '99	547	1.4 ± 0.32	0.15 (0.03–3.88)	6.15 (4)
Imperial	3	July '99	633	1.7 ± 0.27	0.03 (0.009–0.92)	5.32 (5)
Imperial	4	July '99	561	1.1 ± 0.45	1.02 (0.04–10.45)	7.16 (5)
Imperial	5	Aug '99	393	1.5 ± 0.36	0.22 (0.01–4.52)	10.74 (5)
Imperial	6	Aug '99	624	1.6 ± 0.28	0.07 (0.008–1.52)	7.29 (4)
Imperial	7	Aug '99	639	2.0 ± 0.23	0.09 (0.01–1.04)	9.22 (5)
Imperial	8	Sept '99	577	2.1 ± 0.21	1.34 (0.12–6.11)	5.76 (5)
Imperial	9	Sept '99	614	1.7 ± 0.34	2.21 (0.56–6.41)	6.24 (4)
Imperial	10	Sept '99	528	1.5 ± 0.32	0.98 (0.07–4.63)	8.12 (5)
Imperial	11	Nov '99	506	1.8 ± 0.28	0.76 (0.08–3.92)	10.70 (5)
Imperial	12	Nov '99	641	1.4 ± 0.42	0.09 (0.007–1.35)	7.42 (4)
Palo Verde	1	Aug '99	412	1.6 ± 0.38	3.64 (0.72–8.74)	6.12 (5)
Palo Verde	2	Aug '99	572	1.8 ± 0.26	3.45 (0.97–7.22)	7.34 (5)
Palo Verde	3	Sept '99	509	1.5 ± 0.34	4.22 (0.89–9.12)	4.89 (4)
Palo Verde	4	Oct '99	621	2.0 ± 0.22	2.31 (0.91–6.79)	7.53 (5)
Palo Verde	5	Nov '99	563	2.1 ± 0.20	2.98 (1.00–7.32)	8.92 (5)
San Joaquin	1	Sept '99	369	1.7 ± 0.33	1.82 (0.14–7.02)	7.21 (5)
San Joaquin	2	Oct '99	487	1.4 ± 0.40	7.67 (0.91–10.42)	6.18 (5)
San Joaquin	3	Nov '99	439	2.0 ± 0.23	3.28 (1.02–6.74)	9.59 (5)
Yuma	1	Sept '99	497	1.8 ± 0.36	1.91 (0.28–6.77)	10.64 (5)
Yuma	2	Sept '99	546	2.5 ± 0.16	3.20 (0.97–5.92)	7.96 (5)
Yuma	3	Oct '99	601	1.7 ± 0.42	2.87 (0.86–7.20)	8.45 (4)

in California during the three years. This indicates that whiteflies were sensitive in general with the exception that populations from San Joaquin Valley were significantly less susceptible to buprofezin than populations from the desert regions. Intraregional responses of San Joaquin Valley whiteflies in toxicity to buprofezin were significant ($a = 0.05$) in 1997 based on nonoverlapping of their 95% CI (Table 1). During 1997, whiteflies sampled from five sites in San Joaquin Valley represented the least susceptible (LC_{50} = 37.14 mg [lksqb]AI]/liter^{-1}) as well as the most susceptible (LC_{50} = 1.19 mg (AI)/liter^{-1}) of the interregional populations to buprofezin during that season. Toxicity varied by as much as 28-fold between the San Joaquin Valley whiteflies and those of Palo Verde or Imperial Valley (LC_{50}s ranging from 1.31 to 4.57 mg [AI]/liter^{-1}). Although no striking differences were found in whitefly responses from Imperial and Palo Verde Valleys in 1997, the sensitivity of whitefly populations from the Imperial valley to buprofezin increased in 1999 as indicated by the lower LC_{50}s ranging from 0.03 to 2.21 mg (AI)/liter^{-1}. Also the San Joaquin Valley whiteflies represent a significant departure in their responses in 1998 and 1999 compared with the LC_{50}s in 1997 (Toscano et al. 1998).

Results of whitefly responses from the study areas in California were compared with those collected from three to five additional field sites in Yuma region during 1998 and 1999. These results overlapped with buprofezin results obtained within the same year for the Imperial and Palo Verde regions with no significant differences in the LC_{50}s ranging from 1.25 to 3.25 mg (AI)/liter^{-1}.

In the absence of any buprofezin selection, the data indicate that baseline susceptibility levels in whiteflies are likely to vary considerably. This poses an interesting dilemma for future resistance monitoring to the two IGRs. Distinguishing between natural tolerance and genuine resistance (caused by a change in resistance gene frequency due to selection) could therefore prove very difficult.

Slopes of the probit regression estimates were not equal for all locations for the two compounds. The regression lines of most field populations varied from a low of 1.0–2.5 with one exception of 3.5 in August 1998. The lower slopes suggest a high degree of heterogeneity in these populations and probably account for the wide variation in LC_{50}s.

The relative toxicity to buprofezin among all populations sampled in 1997, 1998 and 1999 is represented by the mean ± SEM LC_{50}s in Fig. 1. A comparison of

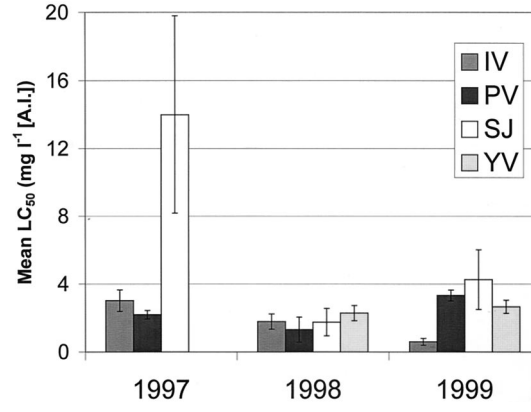

Fig. 1. Mean ± SEM LC_{50}s for buprofezin against nymphs of *B. argentifolii* populations from Imperial Valley (IV), Palo Verde Valley, (PV), and San Joaquin Valley (SJ) in California, and Yuma Valley (YV) in Arizona during 1997 ($F = 2.35$, df = 2, $P < 0.05$), 1998 ($F = 0.42$, df = 3, $P < 0.05$), and 1999 ($F = 11.61$, df = 3, $P < 0.05$).

Table 4. Baseline monitoring of field populations of whiteflies from California to pyriproxyfen in 1997

Location	Field #	Date/Year	*n*	Slope ± SE	LC_{50} (mg [AI]/l^{-1}) (95% CI)	χ^2 (df)
Imperial	1	July '97	1,178	1.9 ± 0.24	5.01 (1.63–11.21)	9.91 (5)
Imperial	2	July '97	752	2.1 ± 0.17	1.56 (0.43–8.06)	8.47 (5)
Imperial	3	Sept '97	414	1.9 ± 0.27	0.94 (0.06–6.78)	6.45 (4)
Imperial	4	Sept '97	566	1.5 ± 0.46	0.61 (0.04–4.29)	8.07 (4)
Imperial	5	Oct '97	635	2.2 ± 0.20	1.17 (0.72–5.84)	7.73 (5)
Palo Verde	1	Aug '97	414	5.9 ± 0.17	10.02 (7.27–12.10)	9.24 (5)
Palo Verde	2	Aug '97	575	2.3 ± 0.45	1.20 (0.19–5.12)	6.60 (4)
Palo Verde	3	Aug '97	632	2.1 ± 0.25	2.51 (0.93–6.43)	10.87 (5)
San Joaquin	1	Aug '97	585	1.9 ± 0.77	0.06 (0.005–1.51)	7.92 (4)
San Joaquin	2	Aug '97	390	1.8 ± 0.21	0.16 (0.04–2.67)	11.29 (5)
San Joaquin	3	Sept '97	620	2.3 ± 0.43	0.03 (0.005–1.01)	9.83 (5)

buprofezin toxicity over the three years showed a definite variation in susceptibility from year to year. The highest mean LC_{50} of 14.0 (±3.93) mg (AI)/liter^{-1} in San Joaquin Valley populations was observed in 1997 but was not significantly different than other whitefly populatons ($F = 2.35$; df = 2, 11; $P = 0.05$). Buprofezin was generally more toxic to all populations in 1998 including the San Joaquin Valley populations (Means ranging from 1.31 to 2.28 (±0.46–0.65) mg (AI)/liter^{-1} showing no significant inter-regional differences ($F = 1.02$; df = 3, 12; $P = 0.05$). Slightly higher LC_{50}s were again observed in San Joaquin Valley whiteflies during 1999 (mean = 4.25 ± 0.68 mg [AI]/liter^{-1}) compared with other whitefly populations. However, the insects from Imperial Valley are significantly more susceptible to buprofezin as indicated by the lowest mean LC_{50} of 0.59 ± 0.34 mg (AI)/liter^{-1} ($F = 11.61$; df = 3, 19; $P = 0.05$) of the inter-regional populations. This indicates a decline in LC_{50}s in 1999 compared with the previous years. The range of susceptibility to buprofezin for Yuma populations did not vary greatly from year to year.

Baseline Susceptibility to Pyriproxyfen. Susceptibility of whitefly eggs to pyriproxyfen from the various locations in California and Arizona between 1997 and 1999 ranged from LC_{50}s of 0.003–9.7 mg (AI)/liter^{-1} (Tables 4–6). This represents a significantly wide range of sensitivity of over 3,000-fold to pyriproxyfen. However, due to high LC_{50}s of four (range, from 4.31 to 10.02 mg [AI]/liter^{-1}) out of 53 fields, this estimate appears skewed from the moderate distribution of the majority of the LC_{50}s which are below 3 mg (AI)/liter^{-1} which gives a 1,047-fold variation instead. For example, whiteflies from one field site in Palo Verde in 1997 showed a high LC_{50} of 10 mg (AI)/liter^{-1} with a similar level of toxicity (LC_{50} = 9.75 mg [AI]/liter^{-1}) in 1998 for a nearby field site. In general, populations from Palo Verde showed higher LC_{50}s in 1997 and 1998 (range, of LC_{50}s from 1.2–10.02 mg [AI]/liter^{-1}) compared with the Imperial or San Joaquin Valley insects (LC_{50}s = 0.03–5.01 mg [AI]/liter^{-1}). San Joaquin Valley insects were the most susceptible to pyriproxyfen during the 3-yr sampling with the lowest LC_{50}s (ranging from 0.03 to 1.27 mg [AI]/liter^{-1}). The confidence limits showed significant statistical differences for pyriproxyfen for Palo Verde whiteflies compared with the other three regions. Despite the wide range in whitefly responses to pyriproxyfen, the toxicity to pyriproxyfen was found to be consistently lower than buprofezin, with the LC_{50}s at least three-fold lower between the least susceptible populations of the two compounds. In this study, pyriproxyfen was observed to have greater ovicidal activity compared with larvicidal activity (unpublished data of N. Prabhaker).

Pyriproxyfen proved very effective against the eggs of the Yuma populations during both years of sampling (Tables 5 and 6). The LC_{50} for the Yuma populations

Table 5. Baseline monitoring of field populations of whiteflies from California and Arizona to pyriproxyfen in 1998

Location	Field #	Date/Year	*n*	Slope ± SE	LC_{50} (mg [AI]/l^{-1}) (95% CI)	χ^2 (df)
Imperial	1	Aug '98	726	2.1 ± 0.27	1.52 (0.45–6.89)	7.95 (5)
Imperial	2	Aug '98	548	1.9 ± 0.32	0.09 (0.006–2.84)	9.23 (5)
Imperial	3	Sept '98	673	2.3 ± 0.19	2.43 (0.87–7.56)	5.94 (4)
Imperial	4	Sept '98	589	2.4 ± 0.21	3.14 (1.24–8.03)	4.85 (4)
Palo Verde	1	Aug '98	2269	2.7 ± 0.05	2.95 (0.75–4.11)	9.20 (4)
Palo Verde	2	Aug '98	701	1.2 ± 0.42	1.17 (0.50–5.42)	8.18 (5)
Palo Verde	3	Oct '98	1772	2.0 ± 0.21	9.75 (7.91–11.67)	4.63 (5)
Palo Verde	4	Oct '98	844	1.7 ± 0.28	3.21 (1.86–4.10)	5.11 (4)
San Joaquin	1	Aug '98	4193	1.6 ± 0.26	1.12 (0.70–3.12)	10.54 (5)
San Joaquin	2	Sept '98	1054	1.3 ± 0.29	1.27 (0.25–3.65)	7.72 (4)
San Joaquin	3	Oct '98	965	1.4 ± 0.28	0.96 (0.17–2.93)	6.34 (4)
Yuma	1	Aug '98	516	1.6 ± 0.21	0.67 (0.09–2.12)	10.23 (5)
Yuma	2	Aug '98	864	1.4 ± 0.34	0.98 (0.32–2.67)	7.35 (4)
Yuma	3	Sept '98	552	1.3 ± 0.23	1.07 (0.27–3.05)	9.71 (5)
Yuma	4	Sept '98	587	1.2 ± 0.41	0.85 (0.21–2.51)	3.83 (4)

Table 6. Baseline monitoring of field populations of whiteflies in California and Arizona to pyriproxyfen in 1999

Location	Field #	Date/Year	*n*	Slope ± SE	LC_{50} (mg [AI]/l^{-1}) (95% CI)	χ^2 (df)
Imperial	1	June '99	455	2.8 ± 0.12	0.72 (0.32–1.88)	8.86 (5)
Imperial	2	June '99	442	2.6 ± 0.18	0.85 (0.36–2.01)	5.82 (4)
Imperial	3	July '99	604	2.3 ± 0.23	0.03 (0.01–0.61)	9.11 (4)
Imperial	4	July '99	562	1.9 ± 0.33	0.05 (0.01–0.82)	10.27 (5)
Imperial	5	Aug '99	734	2.7 ± 0.09	0.87 (0.33–1.06)	6.24 (4)
Imperial	6	Aug '99	806	1.9 ± 0.32	1.06 (0.16–1.51)	9.81 (5)
Imperial	7	Aug '99	762	1.6 ± 0.41	1.51 (0.49–2.46)	7.53 (4)
Imperial	8	Aug '99	796	2.1 ± 0.27	0.74 (0.39–1.83)	6.98 (4)
Imperial	9	Sept '99	824	2.2 ± 0.31	1.36 (0.35–2.21)	6.24 (5)
Imperial	10	Sept '99	673	2.7 ± 0.12	1.48 (0.42–2.05)	10.45 (5)
Imperial	11	Nov '99	836	2.4 ± 0.20	1.19 (0.37–1.99)	5.08 (4)
Imperial	12	Nov '99	922	2.3 ± 0.26	0.16 (0.09–1.44)	9.22 (5)
Palo Verde	1	Aug '99	504	1.6 ± 0.33	4.31 (2.1–26.6)	11.42 (5)
Palo Verde	2	Aug '99	437	1.9 ± 0.29	1.08 (0.22–13.4)	7.76 (4)
Palo Verde	3	Aug '99	526	2.0 ± 0.25	2.94 (1.20–10.36)	9.81 (5)
Palo Verde	4	Sept '99	496	1.6 ± 0.32	1.56 (0.58–7.74)	3.12 (4)
Palo Verde	5	Oct '99	565	1.5 ± 0.49	0.97 (0.13–4.53)	8.24 (5)
Palo Verde	6	Nov '99	624	2.3 ± 0.26	1.28 (0.36–6.54)	5.70 (4)
San Joaquin	1	Sept '99	534	1.8 ± 0.32	0.96 (0.02–1.21)	9.65 (5)
San Joaquin	2	Oct '99	476	2.5 ± 0.21	0.54 (0.12–2.36)	4.52 (4)
San Joaquin	3	Oct '99	528	1.9 ± 0.34	0.61 (0.24–2.53)	7.42 (5)
San Joaquin	4	Nov '99	441	1.6 ± 0.38	0.83 (0.38–2.97)	8.23 (4)
Yuma	1	Sept '99	653	1.7 ± 0.28	1.86 (0.89–3.78)	6.33 (4)
Yuma	2	Sept '99	582	2.2 ± 0.23	0.56 (0.28–2.76)	8.28 (4)
Yuma	3	Oct '99	496	1.5 ± 0.42	0.92 (0.51–3.16)	10.15 (5)

ranged from 0.67 to 1.07 mg (AI)/liter^{-1} in 1998 and in 1999 ranged from 0.56 to 1.8 mg (AI)/liter^{-1}. Whiteflies collected from the various sites in Yuma during the two years of sampling were not significantly variable in their responses to pyriproxyfen. With the exception of Palo Verde whitefly eggs, which were less sensitive to pyriproxyfen, the LC_{50} values of whitefly eggs from Imperial and San Joaquin valleys were not significantly different from the Yuma populations.

The regression lines of the field populations to pyriproxyfen were generally higher compared with the responses exhibited by whiteflies to buprofezin. This may indicate that pyriproxyfen is a more effective compound than buprofezin and elicits a faster response in whiteflies resulting in a range of mortality occurring within a narrow range of concentrations.

Looking at the combined data for 1997–1999, pyriproxyfen appeared to be more effective to whitefly populations in 1999 (Fig. 2). The highest mean LC_{50} was observed in the Palo Verde populations at approximately 5 mg (AI)/liter^{-1} in both 1997 and 1998, but was not significantly higher than the LC_{50}s of most other populations tested ($F = 1.80$; df = 3, 10; $P = < 0.05$). However, the Palo Verde Valley whiteflies were more susceptible in 1999 compared with the two previous years (mean = 2.01 ± 0.32 mg [AI]/liter^{-1}). Whiteflies from the Imperial Valley were more susceptible to pyriproxyfen in 1999 (mean = 0.76 ± 0.21, $F = 3.60$; df = 3, 22; $P = 0.05$).

Discussion

This study was initiated to begin resistance monitoring of field populations of whiteflies from California and Arizona agricultural regions to the newly introduced chemicals, buprofezin and pyriproxyfen (Toscano et al. 1998) under the assumption that resistance genes are absent due to lack of exposure and selection. Laboratory bioassays of field collected populations have clearly shown that immature stages of whiteflies are significantly variable in their responses to the two IGRs. However, despite the variability, there was no significant decrease in overall susceptibility during the study period. In 1997, there was significant intra-regional variation in whiteflies collected from San Joaquin Valley to buprofezin. Populations in Imperial and Palo Verde Valleys did not differ significantly and exhibited levels of sensitivity similar to the most susceptible population of San Joaquin Valley. In 1998 and

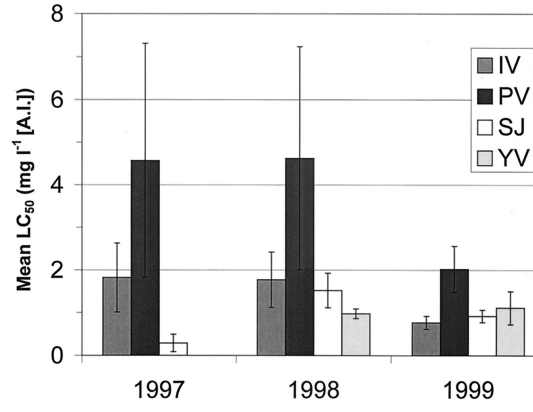

Fig. 2. Mean ± SEM LC_{50}s for pyriproxyfen against nymphs of *B. argentifolii* populations from Imperial Valley (IV), Palo Verde Valley (PV), and San Joaquin Valley (SJ) in California, and Yuma Valley (YV) in Arizona during 1997($F = 1.80$, df = 2, $P < 0.05$), 1998 ($F = 2.91$, df = 3, $P < 0.05$) and 1999 ($F = 3.60$, df = 3, $P < 0.05$).

1999, levels of toxocity were similar in all four regions, although LC_{50}s were slightly lower in 1999. In the case of pyriproxyfen, generally whiteflies from the Palo Verde region of California were least susceptible but were more susceptible to buprofezin.

Identificaton of variations in responses of whiteflies and the dose–response relationships reported in this study through baseline monitoring are now a useful database for continued monitoring in situations where the products are used in control programs. They also provide a foundation for future management of whitefly populations. The potential use of these two selective compounds in the management of whiteflies is encouraging and was demonstrated in managing resistant whiteflies in Arizona (Dennehy and William 1997).

Regional differences in susceptibility to buprofezin have been recorded by several workers (Cahill et al. 1996, Simmons et al. 1997, Yasui et al. 1997, Ellsworth et al. 1999), although the reported differences were generally small in the range of 0.53–6.4 mg/liter^{-1} with two exceptions of greenhouse populations that exhibited higher LC_{50}s of 15.8 (Almeria population) and 24.7 (Netherlands population) mg/liter^{-1}. These differences, although not strikingly higher, may also be associated with the bioassay technique. Different bioassay techniques reveal responses of different magnitude (Dennehy et al. 1983, McCaffery et al. 1988, Shouest and Miller 1991, Prabhaker et al. 1996). The methods selected to determine resistance in field populations to the IGRs should be standardized to permit comparisons of different studies.

An apparent increase in toxicity was observed in 1998 and 1999, indicating an absence of resistance to the two IGRs between August 1997 and November 1999. The absence of a shift toward resistance in whitefly populations suggests a slow adaptation of one or more defense systems in whiteflies. In Israel, three successive applications of pyriproxyfen led to resistance levels as high as 500-fold under greenhouse conditions (Horowitz and Ishaaya 1994). The increased susceptibility to the two IGRs in both the California and Arizona populations probably reflects the judicious use of the two compounds during the last three years.

Although resistance to buprofezin has been reported in whitefly populations from Israel (Cahill et al. 1996, Horowitz and Ishaaya 1994), resistance to the two IGRs has not been reported previously in the populations from United States. A consistent level of susceptibility to buprofezin was observed during 5 yr of monitoring in Arizona with baseline estimates below 11 mg/liter^{-1} (Ellsworth et al. 1999). Our results also confirm the present status of high susceptibility to the two IGRs. In spite of the higher LC_{50}s at some sites in California, resistance in whitefly test populations to buprofezin and pyriproxyfen is not suspected at the present time because these test individuals did not survive the recommended rate of commercial use. All the LC_{50} values reported in this study are significantly lower than the field rates of 2100 and 323 mg (AI)/liter^{-1} for buprofezin and pyriproxyfen respectively. Both IGRs appear to give good control of whiteflies. Therefore, the differences in LC_{50}s observed may be due to a natural variation in tolerance especially at the inter-regional level. Also the previous history of insecticide exposure in each agricultural region may influence the response of whiteflies to the IGRs.

There was no correlation between the toxicities of buprofezin and pyriproxyfen either within or among regional populations. This is an encouraging sign because it suggests the absence of a common mechanism of defense against the two IGRs. Future studies should consider the potential role that resistance to other chemical classes plays with regard to the relative susceptibility of whiteflies to IGRs, because IGRs are considered to be important components of IRM programs both in California and Arizona.

This study included only estimates of lethal effects against the egg or the immatures stages of whiteflies as shown by LC_{50}s to evaluate baseline toxicity of the two IGRs. The importance of sublethal effects was not determined in this study. When compounds such as the IGRs which are slow acting and age specific, are evaluated for toxicity against a target pest, the total effect of both lethal and sublethal effects should be evaluated (Stark and Rangus 1994), as they both play a role in the insect's response. For example, in the case of pyriproxyfen, although the mortality of the eggs was estimated on day 8 after deposition of eggs and application of the insecticide, the subsequent survival of first stage immatures at certain concentrations was not estimated. This could result in an underestimate of the total effect of these two IGRs by avoiding sublethal effects. For our monitoring studies, it seemed sufficient to estimate the effect of the two IGRs against specific stages at a selected time.

Neither buprofezin nor pyriproxyfen are toxic to whitefly adults (Ishaaya et al. 1988). Therefore, it was necessary to measure the effectiveness of these two IGRs against the egg stage or larval survival to adulthood. Previous studies have shown that pyriproxyfen was effective against whitefly eggs (Ishaaya and Horowitz 1992, Horowitz and Ishaaya 1994). With pyriproxyfen, day 8 from the time of egg deposition was chosen as an evaluation period because it normally takes 5–7 d for eggs to hatch. Although we checked the mortality of eggs by the eighth day, the experiment was not terminated until the larvae had developed to the adult stage. Based on this evaluation method, which results in higher LC_{50} values, the selected pyriproxyfen concentrations did not lead to an adaptation toward resistance.

To obtain a realistic picture of field efficacy of the new compounds, both laboratory and field susceptibility tests need to be conducted. Ellsworth et al. (1999) accomplished this by monitoring for susceptibility to buprofezin using methods that allowed evaluation of field developed immatures.

A number of insecticides are available for whitefly control at the present time. However, the availability of two more effective insecticides with different modes of action increases the options available to diversify chemistries within a resistance management

program. The two IGRs have great potential for reducing broad scale insecticide use to that of selective and special use. These compounds are slow acting against a narrow range of sensitive stages of the insect's life cycle and thus potentially reduce selection pressure exerted by the conventional chemistries.

Future studies must include continuous monitoring of buprofezin and pyriproxyfen to track the responses of whitefly populations to these IGRs. If a shift in susceptibility is observed, a resistance management program should be initiated. The best procedure to preserve the effectiveness of new compounds is to minimize selection pressure by resistance management. Careful planning of insecticide use can forestall but not necessarily solve the problem. Even with the best programs of resistance management it is often necessary to shift to new compounds acting on novel targets and then continue with a policy of mimimizing selection pressure. The conclusions from our monitoring survey may have a direct influence on the development of resistance management strategies. Additionally, because each insecticide and the resistance that could potentially develop to that insecticide may have very different properties under different ecological environments, basic information is necessary on the dynamics of resistance development in each region. Therefore, the impact of long-term comparisons of inter-regional monitoring of whiteflies is valuable for developing management programs and will be continued.

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